Both internal and external mechanical force affects postnatal tendon development

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INTRODUCTION: The tendon is critical for transmitting muscle-generated loads to joint movement. The tendon developmental process involves multiple biological organizations from the embryonic to the early postnatal period. Some previous studies showed muscle contraction is essential for tendon differentiation and growth [1,2]. These studies focused on the developmental mechanism of the embryo phase through the analysis of transgenic mice. However, postnatal is also the foundation phase for proper mechanical function [3], and regeneration abilities already exist. [4] Our previous work has demonstrated that major genes, such as Scleraxis, changed dynamically during the embryo and postnatal phase in Achilles tendon development. Especially postnatal tendon development showed a relationship between biological events and developing locomotion ability because Scx expression increased with locomotion changes. [5] These results indicated the importance of the postnatal development phase to understand the detailed mechanism of mechanobiology of tendon development. [6] However, little is known about how postural changes affect tendon development as mechanical stimulation, unlike the embryo phase. In muscle contraction-inhibited models in previous studies, the joint motion by external loading with changing the physical movement may induce the mechanobiological response via passive stretching of the muscle-tendon complex. But there are no studies referred to this point and unveiled yet. Our objective of this study is to explore the roles of internal and external mechanical forces to contribute to postnatal tendon maturation. Utilizing the denervation and arthrodesis model, we made an each-controlled model of both internal muscle contraction and external joint movement with loading mechanical force models. (Fig.1) Our central hypothesis is that the mechanical force generated by muscle contraction and external tension from joint movement with loading promotes tendon maturation in the early postnatal.

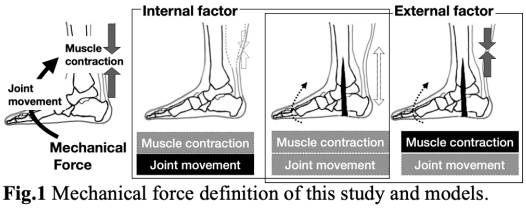

Fig.1 Mechanical force definition of this study and models. DN: denervation, Arth: Ankle Arthrodesis Model, DA: DN+Arth

METHODS: All animal procedures were approved by the Animal Care and Use Committee at Saitama Prefectural University (2021-14). Experimental Design. We divided wild-type C57BL/6 mice into four different models: sciatic nerve denervation model (DN) induced flaccid paralysis, Ankle Arthrodesis Model (Arth) to limit joint movement, both DN and Arth (DA), and Sham in P7 mice. We developed a novel arthrodesis model; Arth placed a 0.15mm diameter stainless needle retrogradely through the calcaneus and across the tibiotalar joint. Achilles tendons were harvested from the post-operative day (d) at 7, and 14. Male and female mice were distributed evenly between groups. The ankle dorsiflexion test. The self-made dorsiflexion equipment with an X-ray device detected the degree of ankle dorsiflexion angle traction by a heavy sinker. Functional Gait. Gait was analyzed by DeepLabCut (DLC) [7] marker-less pose estimation with deep learning and to detect mechanical force for the limb following interventions. Cage activity. Quantity of exercise detected from cage activity before 1 day collect d14 sample using DLC. Gross Image. Zeiss Semi305 captured the whole tendon before each analysis. Histology. The cryosections were used for AlcianBlue/Hematoxylin and Eosin (HE). Mechanical Test. Functional properties of the tendon were assessed using the biomechanical testing system Univert, and tendon cross-sectional area (CSA) was measured from three evenly spaced width and depth measurements from a high-resolution digital camera of both top and side views of the tendon. Transmission electron microscopy (TEM). Ultra-thin sections were stained with 0.2% Oolong Tea Extract/PBS for 30 min followed by lead citrate for 10 min and photographed, and digital images were acquired using EM1010, JEOL, and DegitalMicrograph, Gatan, Inc. Statistics. Statistical analyses included Student's t-test, one-way ANOVA tests followed by Tukey multiple comparison corrections, and Kruskal-Wallis with Dunn's multiple comparisons test.

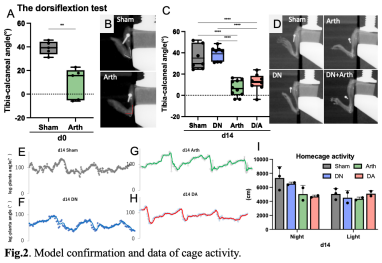

Fig.2. Model confirmation and data of cage activity.

RESULTS SECTION: First, we developed a novel Arth model, and the capacity to inhibit joint movement of Arth was kept between d0 to d14 (Fig.2A-D, n=8-10, $p < 0.05$). Moreover, DN at d14 showed a large dorsiflexion angle during gait (Fig.2E-H). The DN and DA tendon width at d14 was significantly lower than the Sham (Fig.3A, n=4, $P < 0.05$). We observed a decrease in locomotion in the group with the pin inserted compared to Sham (Fig.2E). DN and DA tendon trend to decreased tensile mechanics (Fig.3C). CSA of DN significantly decreased than Sham(n=4, $p<0.05$). TEM images showed DN and DA showed a trend toward an increase in collagen fiber gaps compared to Sham.

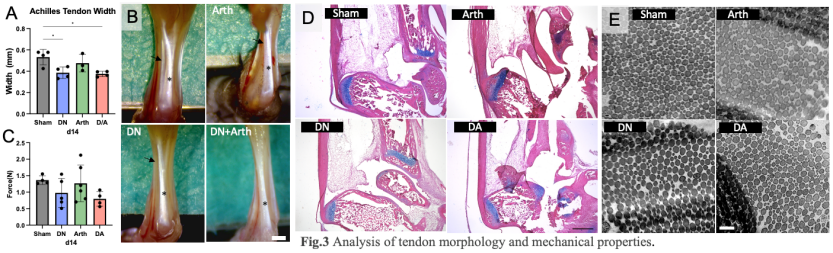

Fig.3 Analysis of tendon morphology and mechanical properties.

DISCUSSION: Our results indicate that both muscle contraction and external force disrupt increasing tendon size and tensile mechanics (Fig.3A-C). External loading has a part of critical role in stimulating tendon development as well, but this effect is smaller than internal loading depending on muscle contraction. Recent studies focused on the postnatal phase are increasing; however, many of them used transgenic techniques for the tendon itself. The tendon development of these animals is affected by two major effects: the direct effect of the deletion of specific genes and the indirect effects of the phenotype of gene editing. This indirect effect is an alteration of physical movement depending on the changed monophony. However, the discussion of transgenic research only referred to the direct effect. In this study, we showed the data of cage activity as the external mechanical stimulation, and the intervention group showed a decreased cage activity. In tendon development, these mechanical effects in the postnatal phase give us the key aspect of tendon maturation.

SIGNIFICANCE/CLINICAL RELEVANCE: An improved understanding of the tendon maturation mechanism during postnatal will be crucial to governing early tenocyte maturation. If tendon mature mechanism induced by mechanical force harnessed therapeutically, such as rehabilitation, it could provide a clue in the clinical treatment of tendon healing or childhood disease.

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